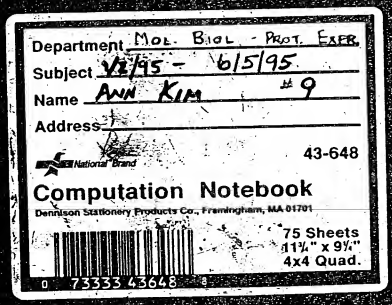


Ruben EXHIBIT #92

Department MOL. BIOL. - PROT. EXPR.

Subject V2/95 - 6/5/95

Name ANN KIM #9

Address

43-648

National Brand

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¼" x 9½"
4x4 Quad.

0 73333 43648 8

HTPAN08 185bp + PQE60

(pg 151 Book 8 #236)

1/24/95

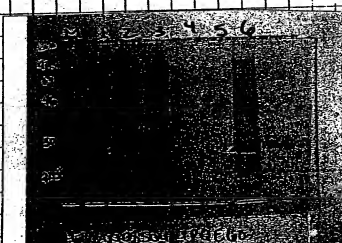
Spin HTPAN08 185 bp + PQE60 in 6M GnHCl pH 8 - 8K 20 min.

Equilibrate Ni SO4 column with pH8 6M GnHCl

Apply Supernatant to Column - Collect Flow
Wash 30 ml pH 8 6M GnHCl - Collect pH8
Wash 30 ml pH 6 6M GnHCl - Collect pH6
Elute 5 ml pH 5 6M GnHCl - Collect pH5
Strip 30 ml pH 2 6M GnHCl - Collect pH 2.

Add 50 µl of [illegible] collected material
to 450 µl H_2O
50 µl 80% 0.15% NaDOC
75 µl 50% TCA.

Mix well
Spin 5 min
Remove supernatant
Resuspend pellet in 15 µl 0.2N NaOH / 15 µl 2X Buff
Heat 100°C. 5 min
Run 20 µl on gel with Rainbow Marker
on 12.5% gel [illegible] at 150V 1 1/2 hrs.
Stain 1/2 hr
De Stain 1/2 hr.



185 bp

1 Uninduced
2 1/ Crude Extract
3 2/ flow
4 3/ pH8
5 4/ pH6
6 5/ pH5
7 6/ pH 2

Dialyze & Send some to protein Exp to renature on column.

Better

1/26/95

V16 PQE60 | HTPAN08 185bp+PQE60

(pg 148 Book 8 #236)

2/2/95

11-6 - Reapply to column + strip again
to try & purify more.
Start Dialyzing ~~[illegible]~~ 20ml in Dialysis
Tubing

3M Gn HCl / Hepes 5hrs.
1.5M Gn HCl / Hepes Overnight.
Reapply 43ml to Ni SO4 Column, to Renature
Over column - of HTPAN08 185bp+PQE60.

2/3/95

Change Buffer
1M Gn HCl / Hepes 6hrs.
0.5M Gn HCl / Hepes Over Weekend.

Carrie will finish

2/6 - 2/10 Vacation!

2/13/95

HTPAN08 185bp + PQE60
Strip Column that has ~~[illegible]~~
renatured - Protein in 2
Strips in Immidazole Elution
Buffer: 50mM NaPO4 pH 6
250 mM Imidizole
300 mM NaCl
10% Glycerol.
2 Strips at 2.5ml each.

Run on Stacking gel with LMW
Marker - 12.5% gel 150V.

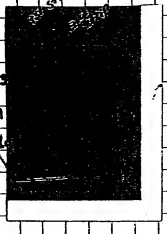
Stain 1/2 hr
destain 1hr.

4 IL-6 PQE60 || HTPA1008 1855bp + PQE60

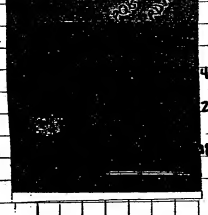
2/3/95

HTPA1008 1855bp + PQE60

Store 4°C.



IL-6 PQE60+PDD 2nd Column Extraction



HTPAN08 / HTPB411 - PD10 - PA2

pg 152 Book 8 # 236

11/26/95

Received Primers for HTPAN08 & HTPB411
Repatect 55°C O/N.

1/27/95

PCR Fragments

HTPB411 PA2

9130 5' Bam.	1
3' Xba New.	1
10x dNTP	50
10x PCR.	50
Taq	2
H_2O	397
DNA (ong/ul)	1
	500ul – 100ul / Reaction.

HTPAN08.

	51 PA2	185 PA2	51 PD10	185 PD10
3146	2	2	2	2
9111:	20	9112: 20	9113: 24	9114: 20
10x dNTP	50	50	50	50
10x PCR	50	50	50	50
Taq	2	2	2	2
H_2O	375	375	371	375
DNA (ong/ul)	1	1	1	1
	500	500	500	500

100 ul / Rxn.

HTPBW08 / HTPB411 PDIO / PAZ

1/27/95

Run PCR:

95°C	5 min	
95°C	30 sec	] 25X
55°C	30 sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold.	

Run 5ul of Rxn on gel with 1kb ladder

1	HTPB411 5' Bam/3' Xba	PAZ
2	HTPBW08 516bp	PAZ
3	↓ 185bp	PAZ
4	516bp	PAZ
5	185bp	PAZ

Precipitate Reactions
Add equal Vol
13% PEG / NaCl
Spin 10 min
Remove Supernatant
Wash pellet 1000ul 70% Ethanol
Drops Rinse Supernatant
Dry pellet 5 min. at R.T.
Resuspend pellet in 100ul TE.
Set up Digests

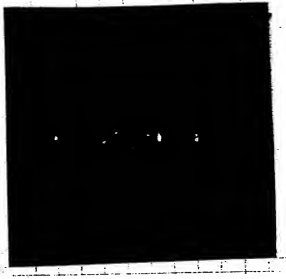
DNA (PCR Rxns)	10 ul
10X #2 Buffer	5
H2O	34
Bam (20)	0.5
Xba	0.5
	50 ul

Incubate 37°C 4hrs.
Run on 0.8% LMP gel with 1kb ladder.

HTPAN08 / HTPB411 PD10 / PA2

1/27/95

Cut out bands
Take picture:



1	HTPAN08	51 bp	PA2
2		185 bp	PA2
3		51 bp	PD10
4		185 bp	PD10
5	HTPB411	PA2	

Gene Clean fragments
- Resuspend in 40ul TE.
Set up ligations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
9111 + 3146	6	6							6								
9112 + 3146			6	6						6							
9113 + 3146					6						6						
9114 + 3146						6						6					
HTPB411 (PA2)							6	6					6				
10 X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	9	9	9	9	9	9	9	9	11	11	11	11	11	15	15	15	17
T4 Lig (1U/ul)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PD10 8/x 1/16					2	2								2			
PA2 8/x 1/16	2		2				2								2		
PA 2 8/x 1/27		2		2				2								2	

		18x
10x Buffer	2	36
T4 ligase	1	18
H_2O	9	162
	12ul	12ul / Tube

Add Appropriate Vector Fragment or H_2O

1/27/95

Incubate ligations 16°C over weekend

16 AR[illegible] 1/27/95

1/30/95

Transform ligations
100ul of Chem. Competent Cells
10ul of ligation Rxn.

PA2 Constructs DH5α ⊕ control PA2
PD10 Constructs M15 rep4 ⊕ control PD10

Incubate on ice 1 hr
Heat 42°C 45 sec
Place on ice
Add 400ul LB
Incubate 37°C OS 1 hr.
Plate 200ul & 300ul onto
LB+Amp plates for all ligations
1-8 onto 150mm plates
for ligations 9-17 plate 100ul
onto LB+Amp 100mm Plates.

Incubate 37°C O/N.

1/31/95

Plates look good
No colonies on ⊖ Control plates
Colonies on ⊕ Control plates

- Inoculate plates colonies onto LB+Amp
for PA2 Constructs-
200ul of LB+Amp in 96 Well dish.

- Inoculate colonies onto LB+Amp Kan
for PD10 constructs
200ul of LB + Kan Amp in 96 Well Dish

1/31/95

B+Amp:

1. 9111/3146 + PA2 9/6 48.
2. 9111/3146 + PA2 1/27 13.
3. 9112/3146 + PA2 9/6. 35.

] Plate # 1

3. 9112/3146 + PA2 9/6 12
4. 9112/3146 + PA2 ~~9~~1/27 13
PA2 9/6 2
PA2 1/27 1
5. HTPB411 + PA2 9/6 48
8. HTPB411 + PA2 1/27 12

] plate #2.

LB+Amp/Kan:

5. 9113/3146 + PD10 48
6. 9114/3146 + PD10 48

] plate #3.

Incubate plate 4 hrs 37°C. with aeration

Setup PCR's.

	1	70x
9111	1	70
3146	0.1	7
10xPCR	3.2	240
10xdNTP	3.2	240
H_2O	22.35	1564.5
Taq	0.15	10.5
Cult.	2	
	32	30 µl/tube

	1	55x
9112	1	55
3146	0.1	5.5
10xPCR	3.2	176
10xdNTP	3.2	176
H_2O	22.35	1229.25
Taq	0.15	8.25
H_2O.	2	
	32	30 µl/tube

		(50x)
9113	1.0	50
3146	0.1	5
10xPCR	3.2	160
10xdNTP	3.2	160
H_2O	22.35	1117.5
Taq	0.15	7.5
Cult	2	
	32	30 µl/tube

		(50x)
9114	1	50
3146	0.1	5
10xPCR	3.2	160
10xdNTP	3.2	160
H_2O	22.35	1117.5
Taq	0.15	7.5
Cult	2	
	32	30 µl/tube

1/31/95

		65X
HTPBY11 5'Bam	0.1	6.5
3'Xba	0.1	6.5
10X dNTP	3.2	208
10X PCR	3.2	208
H_2O	23.25	1511.25
Taq	0.15	9.75
Cult.	2	
	32ul	30ul / tube

PCR.

95°C	5 min	
95°C	20 sec	30X
55°C	20 sec	30X
72°C	1 min	30X
72°C	7 1/2 min	
4°C	Hold.	

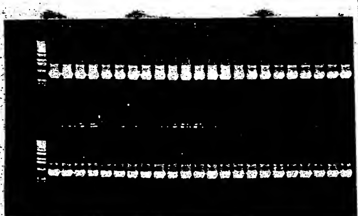
PA2 controls for 9111, 9112, 9 (HTPBY11)

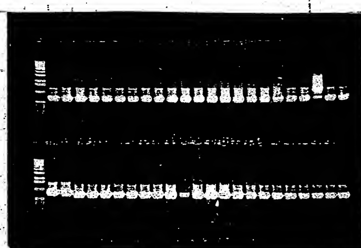
2/1/95

Ran Reactions on 1% TAE Agarose gel with 1Kb ladder.

9113 A1-D12

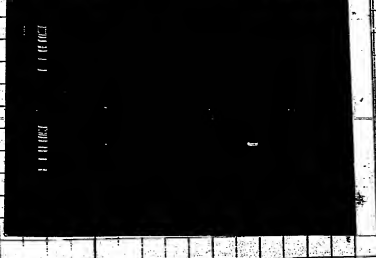
9113/9114 E1-H12





HTPA1008 / HTPB4111 + PD10/P/A2/

2/1/95



12 | HTPA1068 | HTPB411 | PA2 | PD10

1/1/95

Inoculate 200 ml LB + Amp/Kan
with 200 ul of (+) clones to do
small scale inductions (Amicus)
Incubate at 37°C w/aeration
2 hrs.
Add 5 ml of 100 mM IPTG to 2 mM IPTG
final conc.
Incubate 4 hrs 37°C w/aeration
Spin 10 min.
Resuspend pellet in 15 ml H_2O
Add 15 ml 2X Buffer
Store -20°C 4 to tomorrow.

Inoculate 5 ml TB + Amp
w/in PA2 (+) Clones.

(1)	- 11	9N11 / 3126	+ PA2 1/6
(2)	- 8	9N1 / 3146	+ PA2 1/27
(3)	- 10	9N2 / 3146	+ PA2 1/6
(4)	- 10	9N2 / 3146	+ PA2 1/27
(5)	- 1	HTPB411	+ PA2 1/6

Incubate 37°C w/aeration O/N.

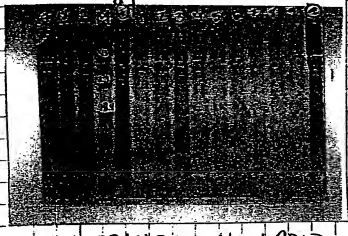
2/2/95

Run Protein gels 15% Stacking.
15 ul of Samples + 1 MW marker.

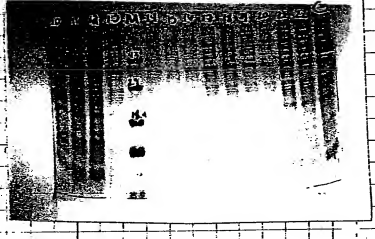
150V 1½ hrs
Stain 30 min 37°C
De Stain 1 hr 37°C

2/2/95

HTPA1508 + PD10



HTPA1508 1585bp + PD10



HTPA1508 1585bp + PD10

HTPA1508 1585bp + PD10

HTPA1508 1585bp

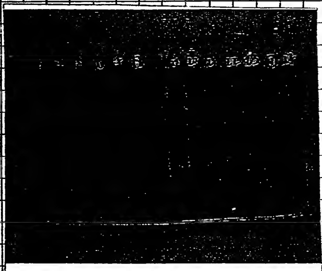
Should produce a protein 32.5KD

HTPA1508 1585bp

Should Produce a protein 27.7 K

Looks like HTPA1508 1585bp + PD10 — Good PCR product. Ready for transformation

2/2/95



looks like all induced except ⊖ control.

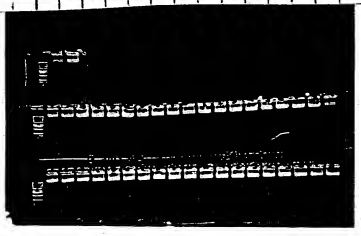
Do large scale prep.

HTPAN08SO4 1850D + PD10

~~[illegible]~~
Do Boiling Mini Preps. of PA2 Constructs

2ml culture Spin 2 min
Remove Supernatant
Resuspend pellet in 750ul STET + RNase + Lysozyme
Heat 100°C 2 min
Spin 10 min
Remove pellet
Add 750 ul 13% PEG-8000 / 1.6M NaCl
Mix well
Spin 10 min
Remove Supernatant
Add 1ml 70% Ethanol to wash pellet
Spin 5 min
Remove Supernatant.
Allow pellet to dry at RT 10 min
Resuspend pellets in 150ul TE
Run 2ul on 1% TAE gel with 1Kb DNA ladder

2/2/95



Mini preps all look good.

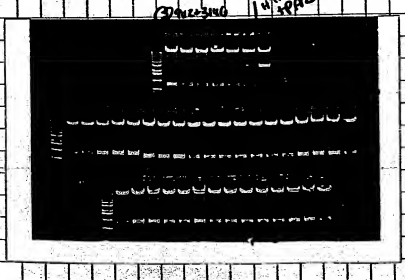
Check for inserts. By digesting w/ Bam / Xba.

DNA	5 µl
10x	3 µl
H_2O	21 µl
Bam	0.1 µl
Xba	0.1 µl
	30 µl

Incubate 37°C overnight

2/3/95

Run 10 µl on gel with 1Kb ladder.



All look correct size. Select 2 HC sequences with internal primers.

①	1, 2
②	1, 2
③	1, 2
④	1, 2

} RPD6 FP16

HTPB411 + PAZ - FP23.

Carrie Checked Seq. Some looked good so did Mini

16 HTPAN08/HTPB411 + PAZ/PD10

1/13/95

- Inoculate 3ml TB+Amp w/ Cultures 1-1, 1-2, 2-1, 2-2, 3-1, 3-2, 4-1, 4-2 of HTPB411+PAZ. Make Glycerols.
- Inoculate 200ml LB+Amp w/ 4ml HTPB411+PAZ to do Maxi Prep
- Inoculate 10ml LB+Amp Kan w/ 1ml of induced culture of HTPAN08/185bp+PD10 (#15) - Do Large Scale induction

1/14/95

- Made Glycerole Stocks + 80°C Protein Expression Box #1
- HTPAN08 185bp + PD10 #12 - Inoculate 300ml LB+Amp/Kan with 3ml of O/N culture. Incubate 37°C 3 hrs - until OD600=0.4-0.6. Add 100mM IPTG @ 2ml - 0.2ml. Incubate 37°C 4½ hrs w/ aeration. Spin 5K 15 min. Resuspend Pellet in 30ml of 0.1M NaPO4 pH8. Store at 4°C O/N.
- HTPB411+PAZ Maxi Prep. Reagent Maxi. Spin culture 6K 20 min. Pour off supernatant.

(pg 27)

HTPAN08 / HTPBY11

(pg 16)

2/16/95

HTPBY11 + PAZ

Resuspend pellet in 10ml of P1 + RNase
Let sit RT 5min
Add 10ml P2 while mixing gently
Add 10ml P3 while mixing
Let sit on ice 20 min
Spin 20 min 8K.
Equilibrate Tip-500 with 10ml QBT
Apply Supernatant to Equilibrated Columns
Wash Column 2X with 30ml QC
Elute DNA in 15ml QF
Add 0.7X (10.5ml) of isopropanol
Mix Well
Spin 9K for 25min
Pour off Supernatant
Wash pellet with ice cold 70% Ethanol (10ml)
Spin 9K 10min
Pour off 70% Ethanol
Allow Pellet to Dry at RT
Resuspend pellet in 400ul TE
Run 1ul on gel
Read OD 260/280 at 1:200 Dilution.

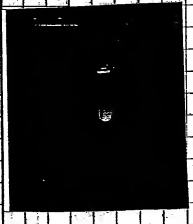
abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
-0.0063	-0.0028	-0.0022	6.9003	0.1449
0.1519	0.0964	0.0232	1.7591	0.5685

1.52ug/ul ≈ 608ug Total

Store 4°C Plasmid Box #2.
Sequence w/ internal primers to confirm sequence

28 HTPB411 & HTPAN108

2/14/95



Probes good.
- See if Sequence is good

2/15/95

Digest @ 1 ug DNA with Bam/Xba to see if insert "Pops" out

DNA (250ng/ul	4
10X #2	3
H_2O	22.6
Bam	0.2
Xba	0.2
	30

Incubate 37°C.

Submit for sequencing w/ internal primers: HTPB411PA2 RP/FP

RP01A	RP06A	RP10	FP14	FP18	FP22	A
RP03A	RP07	RP11	FP15	FP19	FP23	C
RP04A	RP08	FP12	FP16	FP20	RP50	
RP05	RP09	RP13	FP17	FP21	A	

HTPAN08 / HTPBY11 [redacted]

Submitt for Sequencing w/ internals 8/15/95
HTPAN08 51bp + PA2
HTPAN08 185bp + PA2.

Gave Plasmids to Steve to submit to Protein Expression for baculovirus.

51bp.			185bp		
RP12	FP14	RP01	FP16	FP17	RP50.
FP13	RP05	FP18	FP14	RP01	
RP10	FP08	RP06	RP05	FP18	
FP16	FP17	RP50.	FP08	RP06	

HTPAN08PA51RP/FP | HTPAN08P185RP/FP.

HTPAN08 185bp + PD10 #12 large scale ~~Induct~~ Inductions.
Spin Culture 20 min 8K
Prepare NiSO4 Column.
~~Wash~~ Apply 2ml Resin to Column
Wash 20ml of H_2O.
Add 30ml 0.1M $NiSO_4$ to Charge
Wash 30ml H_2O.
Equilabrate with 30ml CoH Gn HCl pH8
Apply Supernatant - Collect Flow
Wash 45ml pH8 - Collect pH8
Wash 45ml pH6 - Collect pH6
Elute 6ml pH5 - Collect pH5
Strip 45ml pH2 - Collect pH2.

Run on 15% Acrylamide Stacking gel.

490ul H_2O
20ul ~~Ecoli~~ GnHCl prep -
75ul 50% TCA
50ul 0.15M NaDOC.

mix Well
Spin 10 min

30 HTPB4II & HTPA008

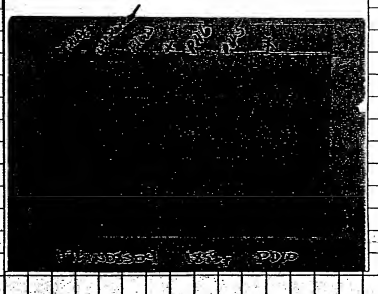
2/15/95

Remove supernatant
Resuspend Pellet 10 µl 0.2N NaOH
Add 10 µl 2x Dissociation Buffer
Boil 5 min - lost pH8 + pH12 < Samples mixed with water.
Spin 5 min
Run 20 µl on gel with LMW Protein Markers
PS 150V 1 hour.

Stain 30 min 37°C
DESTAIN overnight.

2/16/95

HTPA008 185 PDD #12



Protein looks good

Reapply 8ml of pH5.0 to Column
Add 30ml pH8
Send to Protein Expression & have renatured over Column

Immediately 500µl of LPS Amp/Kan HTPB4II + PDD#12
with 20 µl of

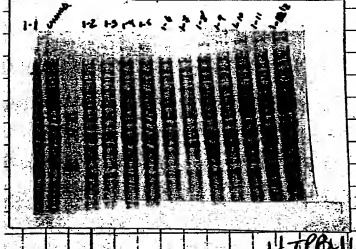
HTPBIII & HTPBD 08

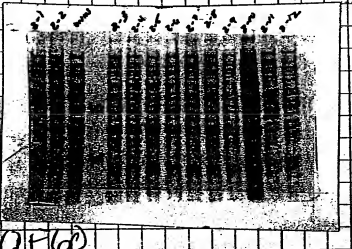
2/16/95

Incubate 37°C w/aeration
2 hrs
Add @ 100 mM IPTG to 2mM
10ml
Incubate 37°C w/ aeration
Overnight.

2/17/95

Spin Cultures 5 min
Remove Supernatant.
Resuspend culture 20 ml H_2O.
Add 20ml 2x Dissociation Buffer.
Heat 100°C 5min
Spin 5 min
Run 10 µl on 10% Stacking
gel
Accidently ran 1 MW Marker instead
of Rainbow Marker.





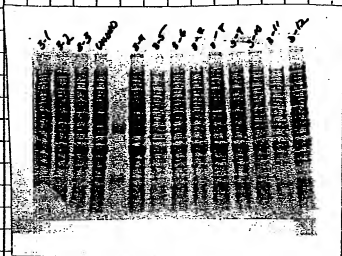
HTPBIII + PQE60

Run 150V 1hr
Stain
DESTAIN

Abe + Rob
2/17/95

 HTPAN08 / HTPBY11

2/17/95



HTPBY11 + PQE60

Try growing up 1 for induction - large scale

2/21/95

Inoculate 5ml LB+ Amp/Kan
with HTPBY11 + PQE60.
2-2 & 3-4
Incubate 37°C

Transform - HTPAN08 5/bp + PD10 1/13/95
HTPAN08 5/bp + PQE60 1/5/95
into M15 Chemically Competent Cells.

Thaw M15 on ice
To 100 µl of Cells add ligations
Incubate on ice 1 hour.

pg 43

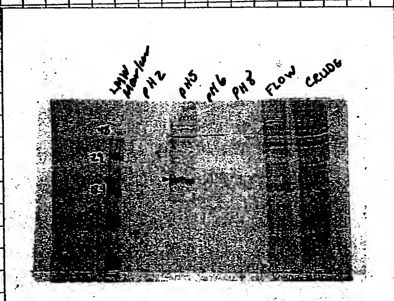
Ni (WT + SV)
(Pg 26)

2/17/95

Mix well
Spin 10min
Remove Supernatant
Resuspend in 10ul 0.2N NaOH
Add 10ul 2X dissociation Buffer
Heat 100°C 5min
Run all on gel.
150V 1 hour.

STAIN 30min 37°C
DESTAIN 30 min 37°C

Take Picture.



looks like I have protein.
Looks slightly Contaminated
Try Ni eluting To try and Clean up prep?
Need [illegible] in PDEGO

2/21/95

pick 48 more clones from the 2/13/95 into 200ul of LB+Amp/Kan

(pg 32)

2/21/95

Heat 42°C 45Sec.
Place on ice
Add 400ul of LB
Incubate 37°C 1 hour.
Plate 300ul onto LB+Amp 150mm plate
100ul onto LB+Amp/Kan 100mm plate
Incubate 37°C O/N

2/22/95

Pick HTPAN08 51 +PQE60 48 BE1-H12
HTPBN08 51 +PD10 48 A1-D12
into 200ul LB+Amp/Kan
Incubate 37°C w/aeration O/N.

HTPB411 +PQE60.
To 300 ml LB+Amp/Kan. add
8 ml of O/N culture. 2.2/3-4
Incubate w/aeration 3 hrs until
OD_{600} ~0.4-0.6
Add 100mM IPTG to 2mM (6ml)
Incubate 37°C 4½ hours
Spin culture 7K 20min
Remove supernatant
Resuspend pellet in 30ml 6M GnHCl
pH8
Store 4°C O/N.

2/23/95

Do PCR of HTPAN08 Clones.

2/23/95

HTPAN08S04 51bp + PD10

		(50X)
9113	1.5	75
3146	0.1	5
10x dNTP	3.2	160
10x PCR	3.2	160
H_2O	21.9	1095
Taq	0.1	5
Culture	2	
		30 µl/tube

HTPAN08S04 51bp + PQE60

		50X
9113	1.5	75
PQE3'	0.1	5
10x dNTP	3.2	160
10x PCR	3.2	160
H_2O	21.9	1095
Taq	0.1	5
Culture	2	
		30 µl/tube

PCR Program # 66

Run 10 µl on gel w/ 1 Kb ladder

HTPAN08 PD10 A1-D12
A2, A7, B12, C5, C7, D5, D9, D10
Inoculate 2 ml LB + Amp/Kan with 20 µl O/N culture

HTPAN08 PQE60 E1-H12
E1, E2, F1, F2, G1, G3, H1, H2
Inoculate 2 ml LB + Amp/Kan with 20 µl O/N culture
Incubate at RT overnight.

HTPBWT8/ HTPB411

2/23/95

HTPB411 + PAE60. 2-2 + 3-4
Spin cultures 5K 10 min
Transfer Supernatant to fresh tube
(Crude Extracts.

Prepare Columns.
Prepare Columns with 2ml NTA Resin
Wash 30ml H_2O
Charge 30ml 0.1M $NiSO_4$
Wash 30 ml H_2O.
Equilibrate 30ml 6M Gn HCl pH8.

Apply Supernatant to Columns.
Collect as flow.
Wash 30ml 6M Gn HCl pH8
Collect as pH8.
Wash 30ml 6M Gn HCl pH6
Collect as pH6.
Elute Protein 6.5ml 6M Gn HCl pH5
Collect as pH5
Strip Columns 30ml 6M Gn HCl pH2
Collect as pH2.
Store 4°C O/N.

2/24/95

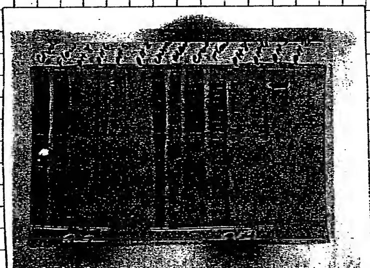
HTPB411 + PQE60.

400ul H_2O
20ul of eluted Protein in 6M Gn HCl.
50ul 0.5% Na DOC.
50ul 50% TCA
Mix well
Spin 5 min.
Remove Supernatant
Resuspend pellet in 0.2N NaOH. ~10ul

 HTPAN586 | HTPB411

7/24/95

Add 10ul 2X Dissociation Buffer
Heat 100°C 5 min
Spin 1 min
Run 15ul on gel with Uninduced
 and Rainbow marker
12.5% Acrylamide Stacking gel
150V 1 hour.



STAIN 37°C
 30 min
DESTAIN 30 min
 37°C

Take Picture

looks like isolated
protein

Store at 4°C
ASK Steve about
 how to get
 [illegible] renature

HTPAN08 51bp + PAE/PD10

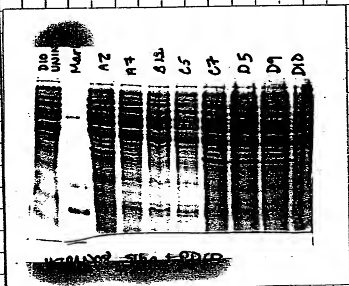
Place tubes at 37°C w/aeration
 for 2 hrs
Add 100mM IPTG to 2mM 4ul
Incubate 37°C 4 hours
Spin 1ml 2 min
Remove supernatant
Resuspend pellet in 30ul H_2O.
Add 30ul 2X Dissociation Buffer
Run 15ul on gel with LMW marker
 150V 1 hour

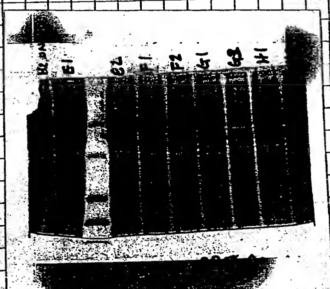
2/24/95

Stain 30 min 37°C
DESTAIN over weekend at RT

2/27/95

Take picture





A2
C7 Induced.
D5
D9 Grow up to make Glycerols. Pick 1 to Do Large Scale Prep.
C7 - large Scale
Inoculate 5ml LB + Amp/Kan w/ C7. Incubate O/N w/ aeration. Make Glycerols of A2, C7, D5, D9

Does not look like anything Induced -)

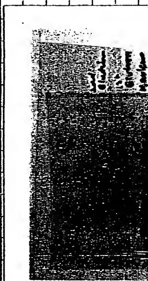
Try Running again - use only 10 µl this time

2/27/95

ReRun HTPAN08 51bp + PQE60
10 µl.

Run 10 µl each of the 1st + 2nd
Imidizole elution of HTPAN08 185 + PD10
#12

2/28/95



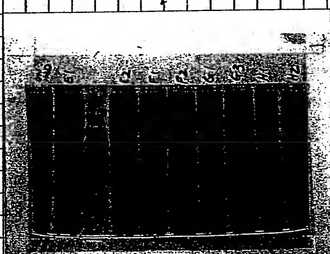
HTPAN08 185bp + PD10 #12

looks like both fractions
have protein.

~~HTPAN08 185bp + PD~~

Inoculate 300 ml LB +
Amp Kan with
HTPAN08 51bp + PD10 #7
to do Qiagen Maxi
Incubate 37°C w/aeration
O/N.

Re Run HTPAN08 51bp + PQE60
150V 1 hour
Stain / DESTAIN



Does not look like
there was any
induction
Try other clones
that lit up

E 4, 5, 6, 8
F 3, 4, 5, 6
G 4, 6, 7, 8
H - B, 4, 5, 9 4, 5, 6, 7

Inoculate 2 ml LB +

2/28/95

Water 20 µl of culture from 4/23 (pg 44).
Let sit at RT O/N

HTPAN08 51bp + PD10 C7 Qiagen Maxi - See pg 42 - ped along side P0160 SV P0E60.

HTPAN08 51bp + PD10 C7
Large Scale Induction
Inoculate 300ml LB + Amp Kan
5 ml of O/N culture.
Incubate 37°C w/ aeration
until OD_{600} = 0.4 - 0.6 ~2½ to 3 hours
Add 100mM IPTG to 2mM (6ml)
Incubate 37°C w/ aeration
4½ hours.
Spin culture 5K 15 min.
Pour off supernatant.
Resuspend pellet in 30ml
6M Gn HCl pH8.
Store 4°C O/N.

3/1/95

Incubate HTPAN08 51bp + P0E60
in 2 ml LB + Amp Kan 37°C
w/ aeration
Incubate until ~ 2 hours
Add 100mM IPTG to 2mM (4µl)
Incubate 37°C 5 hours
Spin 1 ml culture
Remove Supernatant
Resuspend pellet in 40 µl H_2O
Add 40 µl 2X Dissociation Buffer

[signature]
3/1/95

 HTPAN | HTPBY

3/1/95

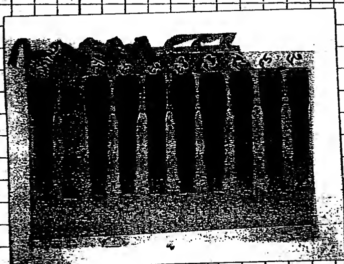
Heat 100°C 5 min
Spin 2 min
Run 10ul on gel w/ 2 MW / Marker
180V 40 min
12.5% gel

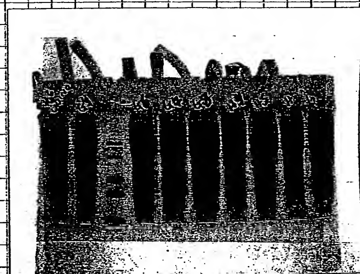
Stain O/N -

~~3/2/95~~
3/2/95

~~DESTAIN~~
to
DESTAIN

Take Picture





Nothing induced -

paged

60 New Clone - HTA Screen

3/7/95

Spin through G25 Column.
Count Lib.

HTAT33	41	1	899759.00	0.21	1.00
HTHCM40	42	1	965819.00	0.20	1.00
HTACB94	43	1	1254146.25	0.20	0.80
HTABG94	44	1	1446598.62	0.20	0.70

Add 100 µl of Salmon Sperm DNA
Boil 100°C 5min
Quick Chill

Pour off Prehyb
Add 10 ml 50% Hyb Buffer
(2 x PIPES / 50% Deionized, 50% Form.)
Add probe to hyb buffer
Incubate 42°C O/N

3/8/95

Wash filters.
Pour off Hyb
Rinse filters 0.2xSSC / 0.1% SDS
Wash 4 filters 65°C
0.2 x SSC / 0.1% SDS.
Wash 3x
Put on film
1-20A - HTSAT33
21-40B - HTABG94
The others leave washing at 65°C.
Not enough Cassettes.

3/9/95

Develop film
Place remaining filters on films
Expose ON at -80°C

K-6SV / HTPAN08 51bp + PDE60

(pg 42) (pg 50)

3/1/95

Add 0.7 Volumes Isopropanol 10.5ml.
Mix well
Spin 8K 30min
Wash pellet 15 ml 80% Ethanol
-20°C.
Spin 8K 10 min
Pour off allow pellet to Dry at RT
20 min.

Resuspend pellet in total of 400ul
TE and transfer to eppendorf
tube.

Read OD260/280 - 1:200 Dilution.

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1 K-6SV PDE60	0.1502	0.0995	0.0219	1.6537	0.6047	1.5ug/ul
2 HTPAN08 51bp PDE60	0.1091	0.0715	0.0144	1.6582	0.6031	1.1ug/ul

Run 2ul on gel w/ 1Kb ladder

Plasmid looks good
Store plasmid #2.
4°C

abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm
0.0942	0.0638	0.0201	1.6947

0.94ug/ul

3/8/95

Start Culture to do an induction of
HTPAN0851bp + PD10

Inoculate 30ml LB Amp Kan with
C-7
Incubate 37°C ON

 H1PAN08 51bp PD ID-

3/9/95

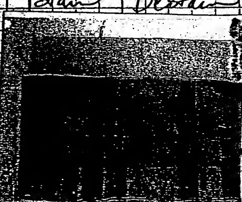
Inoculate 300ml LB+Amp/Kan
10ml of O/N Culture of
H1PAN08 51bp + PD/D BC7
Incubate 37°C w/aeration
Until OD_{600} ~ 0.4-0.6
Add 100mM IPTG to 2mM (6ml)
Incubate 37°C 4 hours.
Spin 8K 20min
Pour off supernatant
Resuspend pellet in 30ml 6M GnHCl pH8
Store 4°C O/N.

3/10/95

Spin Culture 8K 20 min
Transfer Supernatant to fresh tube.
Run on gel:

400ul H_2O
20ul protein in GnHCl
50ul 0.15% NaDOC
75ul 50% TCA.

Mix well
Spin 10 min
Remove Supernatant
Resuspend pellet in 8ul 0.2N NaOH
Run on 8.5% gel.
Stain Destain



looks Good -
- Load Onto
Column

PD 71

pg 40

3/10/94

Develop film for
21A - 40A HT4CM40
1 - 20 B HT4CB44

3/13/94

Pick positive Clones of
HT4A133
HT4CM40
HT4CB44
into 200 ul SM in 96 well dish
Pick 48 from each clone
Incubate Samples at RT O/N

Plate HTA for screening of
HTABG44

Dilute 1:1000 use 20 ul onto
6ml LE392 cells OD600 = 1.0
Incubate 37°C 15 min
Plate onto 150mm NZY Plates with
7ml LB + 0.75% Amp. Let set
Incubate 37°C 5 hours
Store 4°C O/N

3/15/95

for the 48 clones in SM of
HT4A133
HT4CM40
HT4CB44
To 50 ul LE392 OD600 = 1.0 - add 20 ul
of phage
Incubate 37°C 15 min
Add 150 ul NZCYM Broth

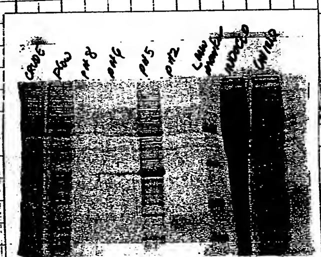
(pg 62)

3/13/95

Prepare NiSO4 Column
2 ml Resin
Wash 30ml H_2O
Strip 20ml 6M Gn HCl pH2
Wash 30ml H_2O
Charge 30ml 0.1M $NiSO_4$
Wash 30ml H_2O
Equilibrate 30ml 6M Gn HCl pH8
Apply supernatant - Collect flow
Wash 30ml pH8 6M Gn HCl - Collect pH8
Wash 30ml 6M Gn HCl pH6 - Collect pH6
Elute 6ml 6M Gn HCl pH5 - Collect
Strip 30ml 6M Gn HCl pH2 - Collect

3/14/95

Run 20 µl on gel with uninduced & induced cultures.



HTPAN08SO4 51bp +PD10

Protein looks good

HTPAN08 51bp+PD10

3/15/95

Prepare 12.5% Preparative gel
1.5 mm

 HTPANO8 886 51bp +PD10

3/15/95

Precipitate 800 ul of pH 5 in H_2O/50% TCA and 0.15% NaDOC
Add 400ul of 0.3N NaOH
Add 400ul 2X Dissociation Buffer
Heat 100°C for 5min
Spin 2min
Run on gel, 100V.

Cut off Marker & Part of gel.

Stain DESTAIN

Align w/ corresponding gel.

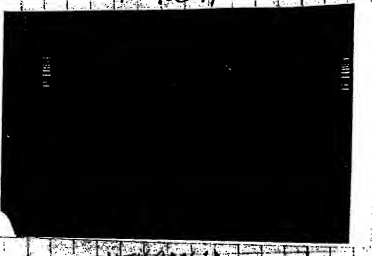
Cut out from unstained gel.
Place in 15ml conical
Ready for Ab Production

pb 133

HT4/HTA screen

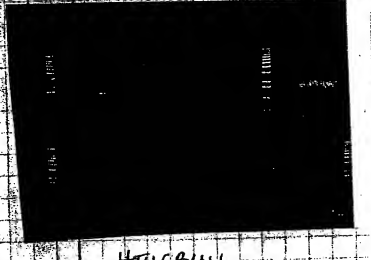
75
3/16/95

HT4CM410

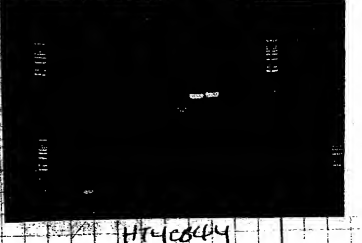


HT4CB414

HT4CM410



HT4CB414



HT4CB414

Wash HTA & HTY filters
0.2xSSC / 0.1%SDS - 3x 65°C
Put on film
-80°C O/N.

3/17/95

Develop film.

Ted M. Ley
3-17-95

Random Prime Probe - HSSEN37, HT4SB02, HSKBN09

4/4/95

Mix by Flicking
Quick Spin
Incubate 37°C 10 min

For HSSEN37 use Prime it

Primers	10ul
DNA	4ul
H_2O	20ul
	34ul

Heat 100°C 5min
Quick Chill
Quick Spin
Add 10ul 5x dCTP Buffer
5ul $\alpha^{32}P$ dCTP
1ul Klenow

Incubate 37°C 10min
put through G 25 column
Count gloves
* Did Not put HT4SB02 HSKBN09
Through Column

CPM	2SIG%	
2391106.50	0.19	HSKBN09
3005574.25	0.19	HT4SB02
1012483.00	0.20	HSSEN37

Add 100ul Salmon Sperm DNA
Heat 100°C 5min
Quick Chill

[signature]
4/6/95

Screen HOAAH / HSSEN / HSKBN / HT4A4 / HT4CB

4/27/95

Inoculate 30 ml TB+Amp
with HTABG94, S01, S02, S03, S04, S05 + S06
Incubate 37°C O/N.

From plated rescues - pick 6 white
colonies into ~~each~~ 200 ul LB+Amp
Incubate 37°C 4 hours
Do PCR

HOAAH62		HSSEN37		HSKBN09	
FP50	1	FP50	1	FP50	2
M13R	0.05	M13R	0.05	M13R	0.05
10X	3.2		3.2		3.2
10X	3.2		3.2		3.2
H_2O	22.4		22.4		21.4
Taq	0.15		0.15		0.15
cult	2		2		2
	32		32		32

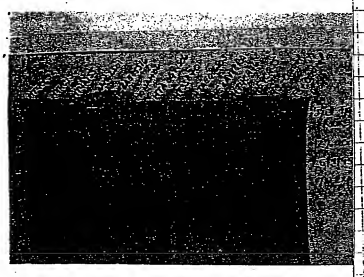
HT4A480		HT4CB44	
FP04	1.2	FP02	1
M13R	0.05		0.05
10X	3.2		3.2
10X	3.2		3.2
H_2O	22.2		22.4
Taq	~~00~~0.15		0.15
Cult.	2		2
	32		32

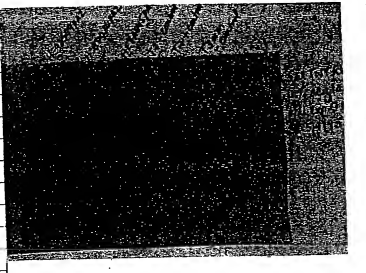
PCR Prog #66

	95°C	5 min
30X	95°C	20 sec
	55°C	20 sec
	72°C	1 min
	72°C	7½ min
	4°C	Hold

[signature]
4/27/95

5/5/95





5/8/95

1972

Inoculate LB+Amp Kan with HTPAW08 51bp/A76 in PDV0 into 100 mL.
Incubate 37°C O/N.

Inoculate 2.50 mL LB+Amp with Plasmid HTABG 94500 for Maxi Prep
Inoculate 30 mL LB+Amp with HOAA116H 50@2

Incubate 37°C O/N W/Vibration

5/9/95

Inoculate 1 loop LB+Amp Kan with 50 mL O/N culture of HTPAW08 51bp A76 in PDV0
Incubate 37°C until OD600 = 0.5-0.6
Add 100 mM IPTG to 2 mM → 20 mL

134 Maxi HTAB6G94SO6 / Midi HOAAH62SO2

5/9/95

Uncoate 37°C 4½ hours
Spin 5K 20 min
Pour off Supernatant
Resuspend pellet in a total of 100ml
10M GnHCl pH 8
Let sit O/N at 4°C

Design Maxi Prep
of HTAB6G94SO6
@ 1:200 Dilution

abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm	280.0 nm
0.1548	0.1071	0.0400	1.7092	0.5851

1.55mg/ml

Run 0.5µl @ on gel

HOAAH62SO2 Alkaline Lysis #1

Spin Culture
Pour off Supernatant
Resuspend pellet in P1 @ + RNase
(from Qiagen) – 2ml
Let sit RT 5min
Add 2ml P2
Mix Gently
Let sit P2 10min
Add 2ml P3
Mix Well
Let sit on ice 10min
Spin 20 min 8K
Transfer Supernatant to fresh tube
Add Isopropanol 0.7 Volumes = 4.2ml
Mix well
Spin 30 min 8K

HTPAN0804 5Lb ATG + PD10

5/9/95

Pour off Supernatant
Wash pellet 70% Ethanol
Spin 10 min 8K
Allow pellet to Dry at RT O/N

5/10/95

HOHAHEZ502
Resuspend pellet in 1ml TE
Transfer to 2 Microfuge tubes
Add equal Volume 13% PEG/1.6M NaCl
Mix well
Spin 15 min
1X 70% Ethanol Wash
Resuspend pellet in a total of 200ul of TE
Run 1% gel
Read OD260/280 1:200 Dilution

OD_{260}	OD_{280}	OD_{320}	$\frac{260}{280}$	$\frac{260}{320}$
0.0279	0.0168	0.0034	1.8322	0.5458

0.2 mg/ml.

[redacted]

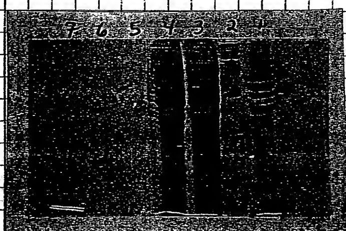
HTPAN08 Strp ATG + PD10.
Spin 8K 20 min
Transfer Supernatant to fresh tube.
Prepare Ni SO4 Column
Pour 3ml Resin into Column
Wash 20ml H2O
Equilibrate 20ml Column Gn HCl pH8
Pour on Supernatant (Crude extract)
and Collect Flow through
Wash Column 60ml Column Gn HCl pH8
Collect
Wash Column - pH8 - 60ml Column 6M Gn HCl pH6
Collect pH6
Elute Protein - 5ml Column Gn HCl pH5
Collect pH5

 HTPAN08504 51bpATG + PD10

5/10/95
Strip Column 30ml 6M GnHCl pH 2.
Collect pH2.

5/11/95

Run Protein Samples on 12.5% Gel
To Samples in 6M GnHCl
450 ul H_2O
50 ul of Sample
50 ul 0.15% Na DOC
75 ul 50% TCA
mix well
Spin 10 min
Remove Supernatant
Resuspend in 10ul @ 0.2N NaOH
Add 10ul 2X Dissociation Buffer
Heat @ 5 min 100°C
Spin
Run Samples on @ SDS-PAGE Stacking gel. 150V 1½ Hours



1 ~~GM~~ - Uninduced
3 ~~GM~~ - Crude Extract
4 ~~5M~~ - flow through
5 ~~6M~~ - pH 8
6 ~~M~~ - pH 6
7 pH5
2 - Rainbow Marker

Does not look good try reinoculating culture and ~~we~~ do another induction

[signature] [signature]

Fragment preps. - Northern Blots

5/11/95

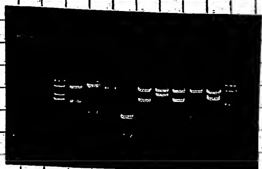
Set up Digests of unknowns to give to Brent Kreider - He'll do Northerns.

Clone ID	Conc	5ug DNA	H2O	10X#2	XhoI	EcoRI
HSKBN09.	2.06ug/ul	2.5	42.1	5ul	0.2	0.2
HNBAA26	0.64ug/ul	7.5	37.1		0.2	0.2
HILBY30	0.5ug/ul	10	34.6			
HT4AI33	PCR Product.	20	24.6			
HT4CM40	0.49ug/ul	11	33.6			
HT4CB44	1.2ug/ul	4.2	40.4			
HNFAA64	0.73ug/ul	6.8	37.8			
HT4A480	0.34ug/ul	15.6	29			
HTABG894	0.99ug/ul	5.2	38.4			

Digest 37°C O/N.

5/12/95

Run 5ul on gel with 1Kb marker



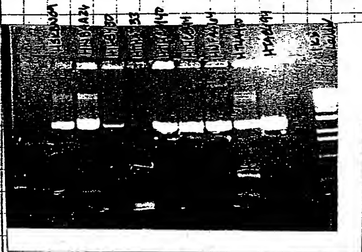
Xho/EcoRI Digests.
1 - HSKBN09.
2 - HNBAA26
3 HILBY30
4 HT4AI33
5 HT4CM40
6 HT4CB44
7 HNFAA64
8 HTA4A480
9 - HTABG94

[signature] 5/12/95

Fragment preps - Northern Blots

5/12/95

Run on 0.8% LMP Gel with 1Kb ladder.
Run 80V 2½ hours.



Cut out fragments
- place into 1.5ml microfuge tube
Store 4°C over weekend.

Need to digest HILB430 again.

HTPAN05 51bp ATG in PD10
- INDUCTION
- Resuspend in 40 ml 6M Gn HCl pH8
- Store 4°C over Weekend.

5/15/95

Gene Clean fragments
Resuspend in 20 µl TE.

Run 1µl on gel with 1Kb ladder

1 HSKBN09
2 HNBAA26
3 HT4A233
4 HTUCM40
5 HT4CB44
6 HNFAA64
7 HT4A480
8 HTABG94
9

Fragments Preps - Northern Blots

5/15/95

Send to Brent Kreider - Kard KDACS

Clone ID	Libraries Expressed	Size in Kb Rt/Xho I	Eco	Approx. Conc(ng/ul)	
HSKBN09	HT4, HSK	~1.4	AMK	~100	15ul
HNBAA26	HBJ, HBM, HCA, HNB, HRG, HTO	~0.800		~100	↓
HILBY30	HIL, HLM	~0.60		WILL SEND 5/17	
HT4AI33	HT4	~0.90		~150	15ul
HT4CM40	HT4	~1.7		~150	
HT4CB44	HT4	~2.5		~150	
HNFAA64	HNF, HSI, HSK, HTA	~1.80		~150	
HT4AY80	HT4, HTX, HT3	~ 0.85		~50	
HTABG94	HTA	~1.7		~150	
HT4CI56	HT4	~1.7		~100	
HT4AI55	HT4	~1.7		~140	
HT4CL32	HT4, HT5, HT3	~1.1		~150	
HT4CA46	HT4, HT3, HCE, HGO, HTA, HL3, HMW	~1.7		~250	
HMSAF22	HMS, HOS, HHF, HSR HTN	~2.0		~100	25ul
HT3SB70	HNF, HT3, HT4, HT5, HTA	~1.5	CLF	~300	5ul
HT4SB02	HET, HGL, HHF, HSU, HT4, HTE, HTP	~1.3	AMK	~200	10ul

Set up Digest of HILB430 PCR product

DNA	20ul
10x H2	5
H2O	24.6
EcoRI	0.2
Xho I	0.2
	50ul

Digest at 37°C overnight

HTPAN08 51 bp ATG + PD10

Put over Column Collect Flow through

pH 8

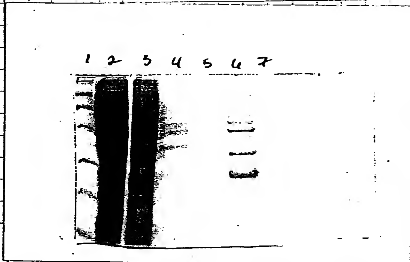
pH 6

pH 5

 HTPAN08 SOU 516bp ATG + PD18

(pg 136)

5/17/95 Run HTPAN08 samples on 12% Acrylamide gel

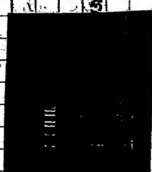


1 Rainbow Marker
2 Crude Extract
3 flow through
4 pH8
5 pH6
6 pH5
7 pH2.

Store at 4°C.

Carrie Did Gene clean of HTLBY30 Xho RI

Run 1ul on gel with 1Kb ladder



Give to Brent
~600 bp
~100ng/ul.

5/18/95

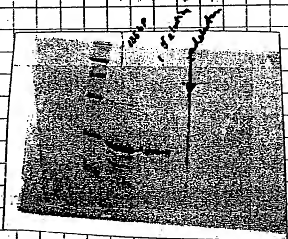
Reapply pH5 of HTPAN0851bpATG to fresh column. (from 3/31/95 - pg 71)
Add 3ml pH5 + 20ml pH8.
Wash 30ml pH8.
Wash 20ml pH6.
Give to protein expression for renaturation over column.

5/19 & 5/22

Computer

5/23/95

Received column Back
Elute 2ml Imidazole elution Buffer. 2 times - Run on 12% Gel with Marker + HTPAN08 51bp ATG



5/23, 5/24, 5/25

Computer

5/26/95

Off.

Pg 28 Book #2115 AMK #10

5/18/95

Reapply pH5 of HTPAN08 51bpATG to fresh Column. (from 3/31/95 - pg 71)
Add 3ml pH5 + 20ml pH8.
Wash 30ml pH8.
Wash 30ml pH6.
Give to protein expression for renaturation over column.

5/19 to 5/22

Competition

5/23/95

Received Column Back
Elute 2ml Imnidazole elution Buffer.
2 times - Run on 12% Gel
with Marker + HTPAN08 51bpATG

5/23, 5/24
5/25

Competition

5/26/95

Off.

Pg 28
Book #7115
AMX #10